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DO REPRODUCTIVE HISTORY TRAITS RELATE TO SEED MATURATION IN A CLONAL HERB?

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ABSTRACT. A field experiment tested whether seed maturation in Canada mayflower (*Maianthemum canadense*) was pollen limited and whether ramets flowering for the first time differed from ramets flowering for the second time in the number of seeds that they matured. Addition of pollen increased the number of seeds matured by ramets and therefore seed maturation was pollen limited. No difference in the number of seeds matured by ramets flowering for the first time and ramets flowering for the second time was detected and thus previous flowering did not appear to affect current seed maturation. There was a positive relationship between the number of seeds matured by ramets and the total weight of these seeds. This relationship did not differ between ramets flowering for the first and second times.

Key Words: ramet, genet, Canada mayflower, *Maianthemum canadense*

Plants must allocate limited resources to structures associated with growth, defense, and reproduction (Bazzaz et al. 1987). Over a plant's lifetime, allocation patterns must balance survivorship and fecundity (Bazzaz et al. 1987).

A tradeoff between current fecundity and subsequent growth and fecundity has been reported for the orchids *Cypripedium acaule* (Primack and Hall 1990), *Epidendrum ciliare* (Ackerman and Montalvo 1990), and *Tipularia discolor* (Snow and Whigham 1989). Current fecundity also reduced growth in mayapple (*Podophyllum peltatum*; Sohn and Policansky 1977, although see Benner and Watson 1989) and reduced the probability of producing females in jack-in-the-pulpit (*Arisaema triphyllum*; Bierzychudek 1984).

Current fecundity in self-incompatible, insect-pollinated plants may be influenced by a number of variables including the availability of compatible pollen (Bierzychudek 1981; Thompson and Stewart 1981) and also by a plant's reproductive history. This is not to suggest that a plant shown to be pollen limited in a given year is not ultimately resource limited (Bierzychudek 1981) as the addition of pollen in one year may increase current fecundity at the expense of future fecundity (Janzen et al. 1980).

Canada mayflower (*Maianthemum canadense* Desf.; Gleason and Cronquist 1991) is a rhizomatous, perennial herb with genets that consist of dimorphic ramets. Flowering ramets have 2–3 leaves with a terminal inflorescence consisting of 4–35 perfect flowers, while vegetative ramets have only one leaf. Fruits contain 1–4 seeds. Canada mayflower is self-incompatible (Worthen and Stiles 1986).

In the fall, ramets "die back," leaving an overwintering bud that will become the ramet in the following year. At this time it is possible to determine "by touch" whether a bud is vegetative or flowering (Ganger 1998). The status of this bud appears to be determined much earlier, and flower primordia are visible under the microscope as early as May (Kana 1982). With the dieback of the ramets, an abscission scar is left. The scars left by vegetative and flowering ramets are distinctive so it is possible to determine the age of a ramet and whether this ramet has been vegetative or flowering for each of its previous years (Ganger 1997; Silva et al. 1982). It is possible to make this determination in the field with a hand lens, but not as reliably as with a dissecting scope in the lab. It is sometimes possible to identify a ramet that has flowered previously by the presence of a dead flowering stalk.

The flowering ramets of Canada mayflower vary with respect to the number of times they have flowered previously and the number of years spent vegetative prior to flowering or since flowering (Ganger 1997). It is not known whether ramets that remain vegetative longer mature more seeds. It is also not known whether ramets experience a cost of previous flowering. If there were such a cost, then ramets flowering for the second time may mature fewer seeds than ramets flowering for the first time. This cost may also be reflected in the resources allocated to these seeds. Specifically, previously flowering ramets may allocate fewer resources to a similar number of seeds than other ramets.

A field experiment was undertaken to address the following questions: (1) Do ramets that remain vegetative for a longer period of time mature more seeds? (2) Is there evidence of a cost of flowering such that ramets flowering for the first time mature more seeds than ramets flowering for the second time? (3) Is

Canada mayflower pollen limited in local populations? (4) Is there a relationship between the number of seeds matured by ramets and the total weight of these seeds? and (5) Does this relationship differ between ramets flowering for the first and second time?

MATERIALS AND METHODS

Field work was conducted in a mixed coniferous-hardwood forest in the University of New Hampshire woodlands, Durham, NH. Sixty ramets that were flowering for the first time were identified as well as 60 ramets that were flowering for the second time. Ramets flowering for the first and second time occurred together, often within millimeters of one another. These two "flowering" treatments represented one of the factors in a two-factor experiment. Seed maturation in Canada mayflower has been demonstrated to be pollen limited in New Jersey populations (Worthen and Stiles 1988) and in previous years in the University of New Hampshire woodlands (Ganger 1997). Therefore, a second factor, "pollination," was included. Half of the flowering ramets in each of these treatments were randomly assigned to either of two treatments: hand pollinated or open pollinated. Including this factor reduced the likelihood that differences in seed maturation between first- and second-time flowering ramets would be obscured by low levels of pollination.

Each of the flowers of the hand-pollinated ramets were pollinated each day for the life of the flowers. Pollen was collected immediately prior to use from 10–20 flowering ramets not more than 20 m away and applied to stigmas with a wooden toothpick. Flowers from the open-pollinated ramets were not manipulated and presumably received natural levels of pollination. At the end of the fruiting season, all of the flowering ramets were excavated and taken to the laboratory. The numbers of fruits and seeds matured by each ramet was noted. Seeds were removed by gently pushing on the fruits. Seeds were dried for 72 hours at 80°C in a drying oven and then weighed to the nearest 1×10^{-5} g using a Mettler AE 63 balance. The total weight of all seeds matured by ramets was calculated. For each of the ramets that flowered for the first time, the number of years prior to flowering was noted. For each of the ramets that flowered for the second time, the number of years since flowering was noted.

In order to determine if ramets that remained vegetative for a longer period of time were able to mature more seeds, two regressions were performed. For hand-pollinated, first-time flowering ramets, the number of seeds they matured was regressed on the number of years prior to flowering. For hand-pollinated, second-time flowering ramets, the number of seeds that they matured was regressed on the number of years since flowering.

If ramets that were flowering for the first and second time differed in the amount of resources that were allocated to seed maturation, then this difference may have been apparent either as a difference in the number of seeds matured or as a difference between the two types of ramets, in the resources allocated to a similar number of seeds.

In order to test whether the number of seeds matured by ramets flowering for the first time differed from ramets flowering for the second time and whether seed maturation in ramets overall was pollen limited, a two-way analysis of variance (ANOVA) was performed. The statistical model consisted of two factors: flowering (first-time flowering vs. second-time flowering ramets) and pollination (hand pollinated vs. open pollinated). Both flowering and pollination were fixed, categorical variables. The dependent variable was the number of seeds matured. If there was a difference in the number of seeds matured between ramets flowering for the first and second time, then this effect would more likely be observed in ramets that received an overabundance of pollen. In other words, if resource limitation was occurring to a greater extent in either the first- or second-time flowering ramets, then the difference in seed maturation would more likely be detected statistically if pollen-limited ramets were not included. Therefore one *a priori* contrast was considered: the number of seeds matured by hand-pollinated, first-time flowering ramets was compared to the number of seeds matured by hand-pollinated, second-time flowering ramets.

In order to determine if ramets that were flowering for the first and second time differed in their allocation of resources to seeds, two analyses of covariance (ANCOVA) were performed. ANCOVA allowed for a determination of whether there was a relationship between the number of seeds matured by ramets and the total weight of these seeds, and whether this relationship varied between first- and second-time flowering ramets. Two separate ANCOVAs were performed, one for open-pollinated ramets and another for hand-pollinated ramets. In order to determine what variables influenced seed maturation, the number of seeds was the dependent variable. The number of times flowering was the independent, categorical variable and the total weight of seeds per ramet was the covariate. In these analyses, only those ramets that matured seeds were considered.

RESULTS

Forty-six of 60 open-pollinated ramets matured one or more seeds while 51 of 60 hand-pollinated ramets matured one or more seeds. Six ramets were excluded from the analyses because they were found to have flowered more than twice, they could not be accurately aged, or their reproductive history could not be determined due to decay.

The average number of years prior to flowering for ramets flowering for the first time was 3.9 years (SD = 1.81) and thus the average age of these ramets was 4.9 years (SD = 1.81). For ramets flowering for the second time, the average number of years since flowering was 2.9 years (SD = 0.75) and the average age of these ramets was 8.4 years (SD = 1.87). The number of seeds matured by hand-pollinated ramets was not correlated with the number of years prior to flowering for first-time flowering ramets ($F_{1,20,0.05} = 0.53$, $p = 0.47$) nor with the number of years since flowering for second-time flowering ramets ($F_{1,28,0.05} = 2.34$, $p = 0.14$).

Ramets that were hand pollinated matured more seeds than ramets that were open pollinated ($F_{1,107,0.05} = 127.22$, $p < 0.005$, $r^2 = 0.11$; Figure 1). There was no difference in the number of seeds matured by ramets flowering for the first and second time ($F_{1,107,0.05} = 0.50$, $p = 0.48$; Figure 1). There was no interaction between the pollination and flowering factors ($F_{1,107,0.05} = 0.90$, $p = 0.35$). The *a priori* contrast was also not significant; there was no difference between the number of seeds matured by hand-pollinated, first-time flowering ramets and hand-pollinated, second-time flowering ramets ($F_{1,106,0.05} = 0.63$, $p = 0.43$; Figure 1). In concluding that there was no difference in the number of seeds matured between these two treatments, there is an associated probability of being wrong. This is Type II error or β. Following Winer et al. (1991) it was possible to determine β and therefore the statistical power $(1 - \beta)$ of this comparison, given specific

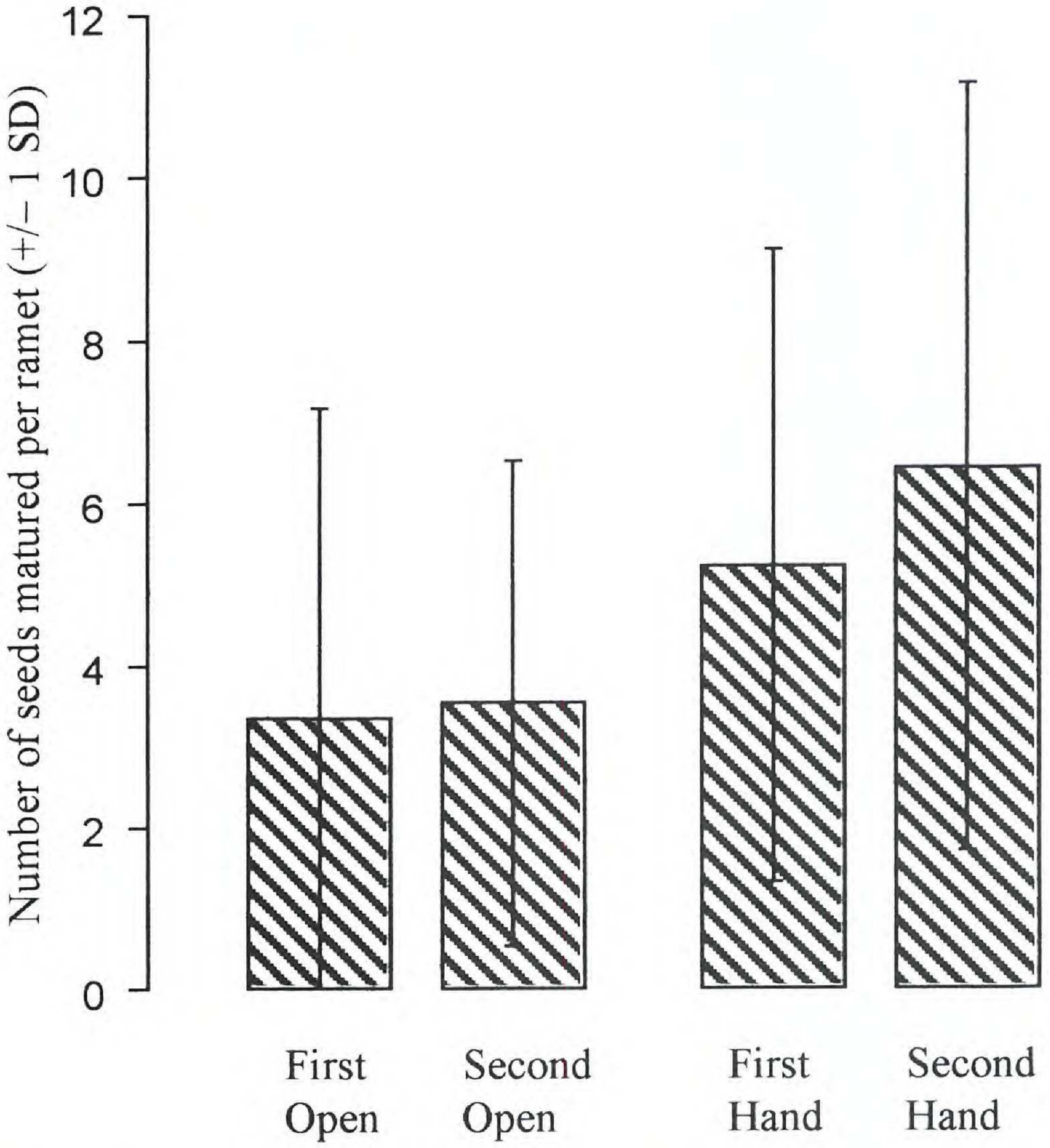

Figure 1. Mean number of seeds matured by *Maianthemum canadense* ramets in each of the four experimental treatments. First = first-time flowering ramets; Second = second-time flowering ramets; Open = open-pollinated ramets; and Hand = hand-pollinated ramets.

alternative hypotheses. The statistical power of the test, assuming a true difference between the treatment means of 1, 2, and 3 seeds, was determined to be 0.21, 0.51, and 0.80, respectively.

Seeds varied in weight from 3 to 18 mg. For open-pollinated, first- and second-time flowering ramets, there was no difference in the relationship between the number of seeds matured and the total weight of these seeds with respect to either the slopes of these relationships ($F_{1,40,0.05} = 1.37$, $p = 0.25$; Figure 2A) or the y-intercepts ($F_{1,41,0.05} = 1.89$, $p = 0.17$; Figure 2A). There was a positive relationship between the number of seeds matured and

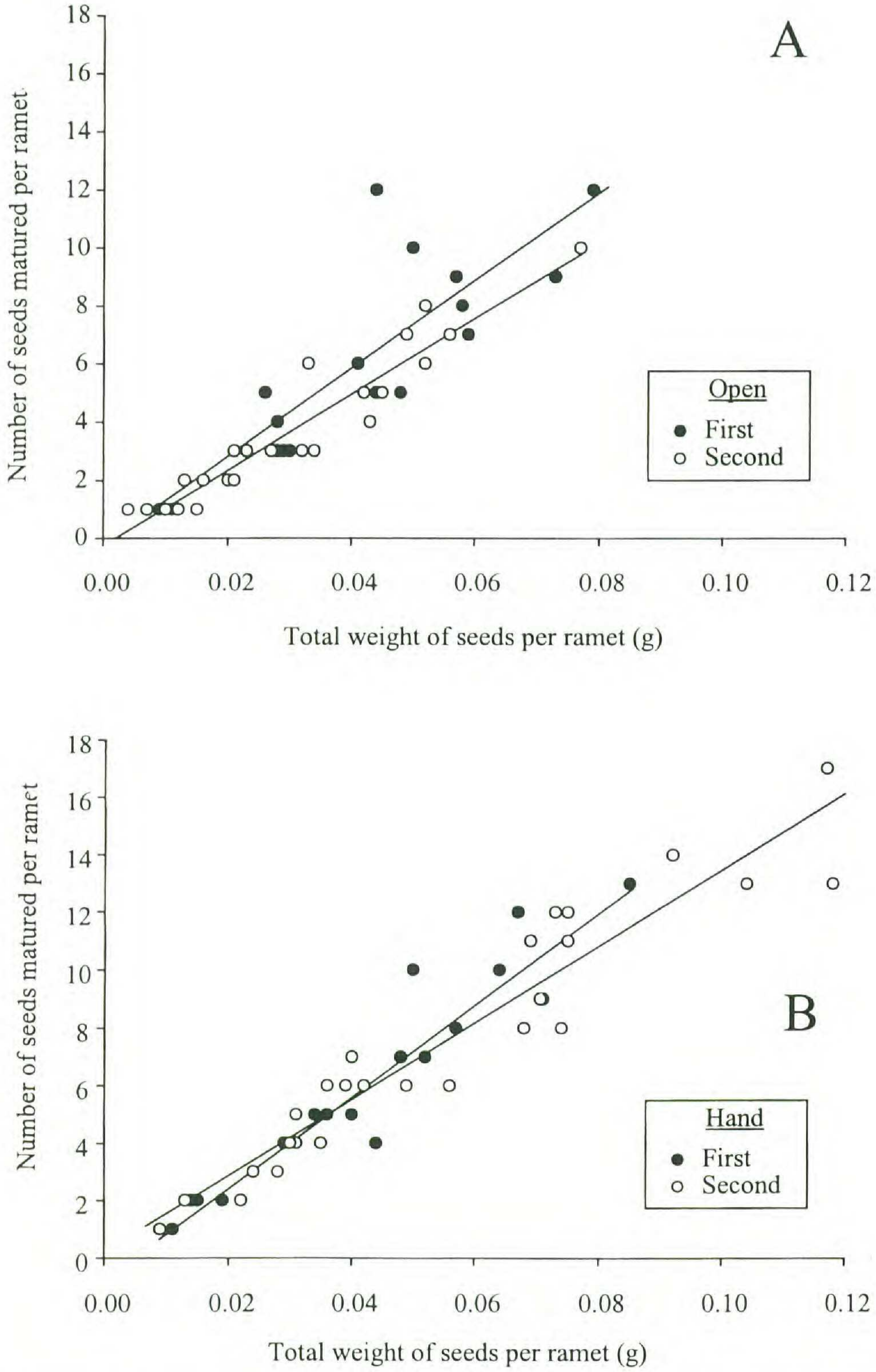

Figure 2. Plots of the number of seeds matured by *Maianthemum canadense* ramets and the total weight of these seeds for (A) open-pollinated ramets and (B) hand-pollinated ramets. First = first-time flowering ramets; Second = second-time flowering ramets; Open = open-pollinated ramets; and Hand = hand-pollinated ramets.

the total weight of these seeds ($F_{1,40,0.05} = 176.25$, $p < 0.001$, $r^2 = 0.83$). For hand-pollinated, first- and second-time flowering ramets, there was no difference in the relationship between the number of seeds matured and the total weight of these seeds with respect to the slopes ($F_{1,41,0.05} = 2.57$, $p = 0.12$; Figure 2B) or the y-intercepts ($F_{1,42,0.05} = 0.55$, $p = 0.46$; Figure 2B). There was a relationship between the number of seeds matured and the total weight of these seeds ($F_{1,41,0.05} = 290.78$, $p < 0.001$, $r^2 = 0.90$).

Seed number was used as the dependent variable in both the ANOVA and the ANCOVAs in order to focus on what factors influenced seed maturation in Canada mayflower. It would also be valid in the ANCOVAs to use seed number as the independent variable and the total weight of seeds per ramet as the dependent variable. This, however, would change the focus from what factors influenced seed maturation to what influence did seed number have on seed weights. The results of such analyses did not differ from those presented above and this is likely due to an overall correlation between seed number and the total weight of seeds ($p < 0.001$, $r^2 = 0.92$).

DISCUSSION

The number of years prior to flowering for first-time flowering ramets and the number of years since flowering for second-time flowering ramets were not related to the number of seeds matured by ramets. Therefore ramets that waited longer to flower did not necessarily mature more seeds. Watson (1984, 1990) has referred to ramets as "mouths" which accumulate resources for use by the genet and for the ramet itself in the future. It is possible that with Canada mayflower, a vegetative ramet continues to accumulate resources until a specific threshold is reached and that this triggers the transition to a flowering ramet. The number of years since flowering may then be related to two variables: (1) the cost incurred by the ramet for past flowering and seed maturation (this may be influenced by the degree of ramet integration) and (2) the quality of the habitat. In other words, a ramet that waited a greater number of years to flower again may have incurred a great cost of previous reproduction, one that the genet was not able to offset, or the quality of the habitat was such that resources were able to be replenished only very slowly, or even some combination of the two. It may also be that another part of the genet was flowering during the intervening years between flowering and that the ramet in question was subsidizing the seed maturation of another part of the genet.

Ramets matured comparable numbers of seeds regardless of whether this was their first- or second-time flowering. Thus the act of flowering did not appear to influence the success of future seed maturation. This was true as well for ramets that received an overabundance of pollen. For this contrast, statistical power was low for a hypothesized difference of one seed. However, at a hypothesized difference of three seeds (approximately the difference between the pollination treatment means) power was quite high.

The addition of pollen resulted in an increase of, on average, 2.7 seeds per ramet. This suggests that seed maturation in ramets was pollen limited during the experiment. Moreover, the addition of pollen had a similar effect on ramets flowering for the first and second time.

There was a strong relationship between the number of seeds matured and the total weight of these seeds for both open- and hand-pollinated ramets. Furthermore this relationship did not differ between ramets flowering for the first and second time. This is further evidence that there was not a cost of having flowered in the past.

While it was possible to determine if ramets had previously flowered, nothing is known about the success of previous flowering. It could be that seed maturation in the past was low and therefore the costs incurred by ramets at that time was also low. The cost of having flowered and reproduced in the past may also have been mitigated by other ramets at the time of flowering. Canada mayflower ramets are known to have increased seed maturation if they receive ample pollen and their rhizomes are left intact, but not if ramets receive ample pollen and their rhizomes are severed (Ganger 1997). This suggests that resources may be translocated to the flowering ramet, either from the rhizome or from other ramets, and may offset the cost of reproduction incurred by the ramet. It is important to note that second-time flowering ramets are much rarer than first-time flowering ramets and that second-time flowering ramets are a subset of ramets that have flowered before. Therefore these results should be viewed with caution.

Canada mayflower is not the only example of a plant in which a cost of previous flowering was not detected. In fact some plants show an increased likelihood of future flowering with greater current reproductive allocation. In the orchid *Spiranthes cernua,* Antlfinger and Wendel (1997) found that individuals producing fewer flowers were less likely to flower in the following year than individuals producing more flowers. This was despite the fact that individuals producing many flowers had a tendency to decrease in size in the following year. In the early spider orchid *Ophrys sphegodes,* Hutchings (1987) found that individuals flowering in the current year were: (1) more likely to flower in the following year than either vegetative or dormant individuals, (2) less likely to enter dormancy than either vegetative or dormant individuals, and (3) had greater survivorships than dormant individuals.

Other species show no effect of current flowering on future reproduction. Horvitz and Schemske (1988) experimentally created high and low levels of reproductive effort in *Calathea ovandensis* and found no difference in the growth, survival, and reproduction of individuals between treatments in the following year. Smith and Young (1982) found that individuals of *Senecio keniodendron* that had high levels of reproduction were more likely to die than other individuals. However those that did survive had higher levels of reproduction than other individuals in the following year.

The notion that previous reproduction should negatively affect present reproduction seems plausible. Since in plants, size and reproduction and size and survivorship tend to be positively correlated, allocation of resources to reproduction in the current year may not only reduce growth in the current year, but may also reduce future fecundity and survivorship due to decreased size (Lovett Doust 1989; Stephenson 1981). This relationship in plants such as Canada mayflower appears to be difficult to establish, in part because of the ability of Canada mayflower to be vegetative for a number of years between flowering and also due to Canada mayflower's clonal habit. These would allow Canada mayflower ramets to mitigate the cost of previous and current reproduction. It is possible then that the cost of previous reproduction, while not apparent at the level of the ramet, would be evident at the level of the genet.

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